Potency Assays for Existing Vaccines

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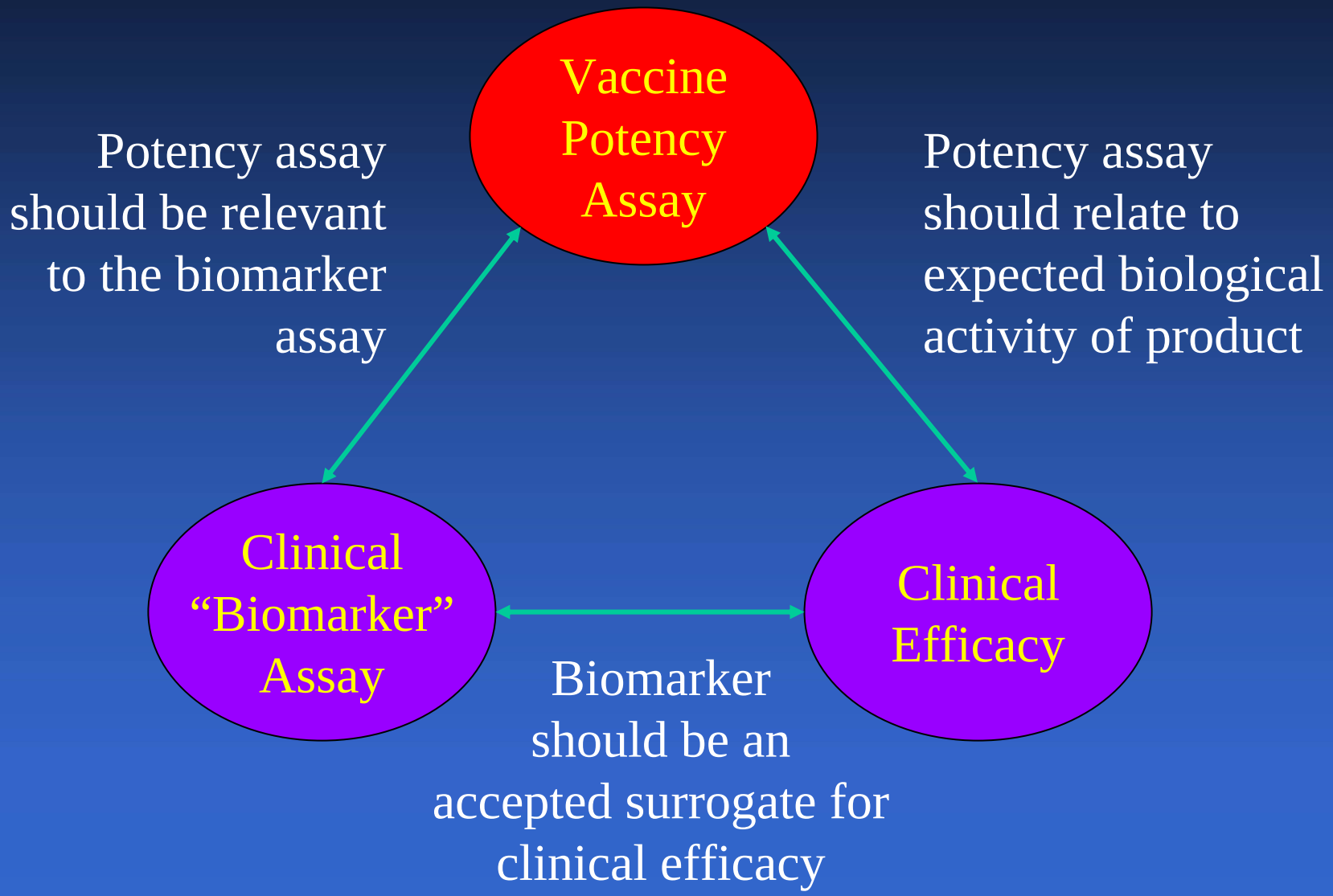
Assaying Potency of Novel Vaccines Oct. 11 – 12, 2005

Potency: what are we after?



A Limited Survey of Licensed Vaccines

- US 52 vaccines licensed (CBER web site)
  - at least 4 more currently under review
  - 28 product types
    - 3 new types under review

- EU 13 vaccines centrally licensed (EMEA web site)
  - at least 3 more currently under review
  - 28 product types licensed in UK (MHRA web site)
    - 3 new types under review

A Limited Survey of Licensed Vaccines

- Licensed (and under review) vaccines cover 24 different pathogens (59 if all serotypes counted)
  - 14 viral (24 types)
    - Most attenuated; some inactivated/recombinant
  - 10 bacterial (35 types)
    - Most purified components; some attenuated

A Limited Survey of Licensed Vaccines

- Licensed (and under review) vaccines cover 24 different pathogens (59 if all serotypes counted)
  - 14 viral (24 types)
    - measles
    - mumps
    - rubella
    - polio# (3)
    - rotavirus (5)*
    - influenza# (2)
    - rabies#
    - varicella
    - small pox#
    - human papilloma virus# (4)*
    - hepatitis A#
    - hepatitis B#
    - Japanese encephalitis virus#
    - yellow fever virus#
  - 10 bacterial (35 types)

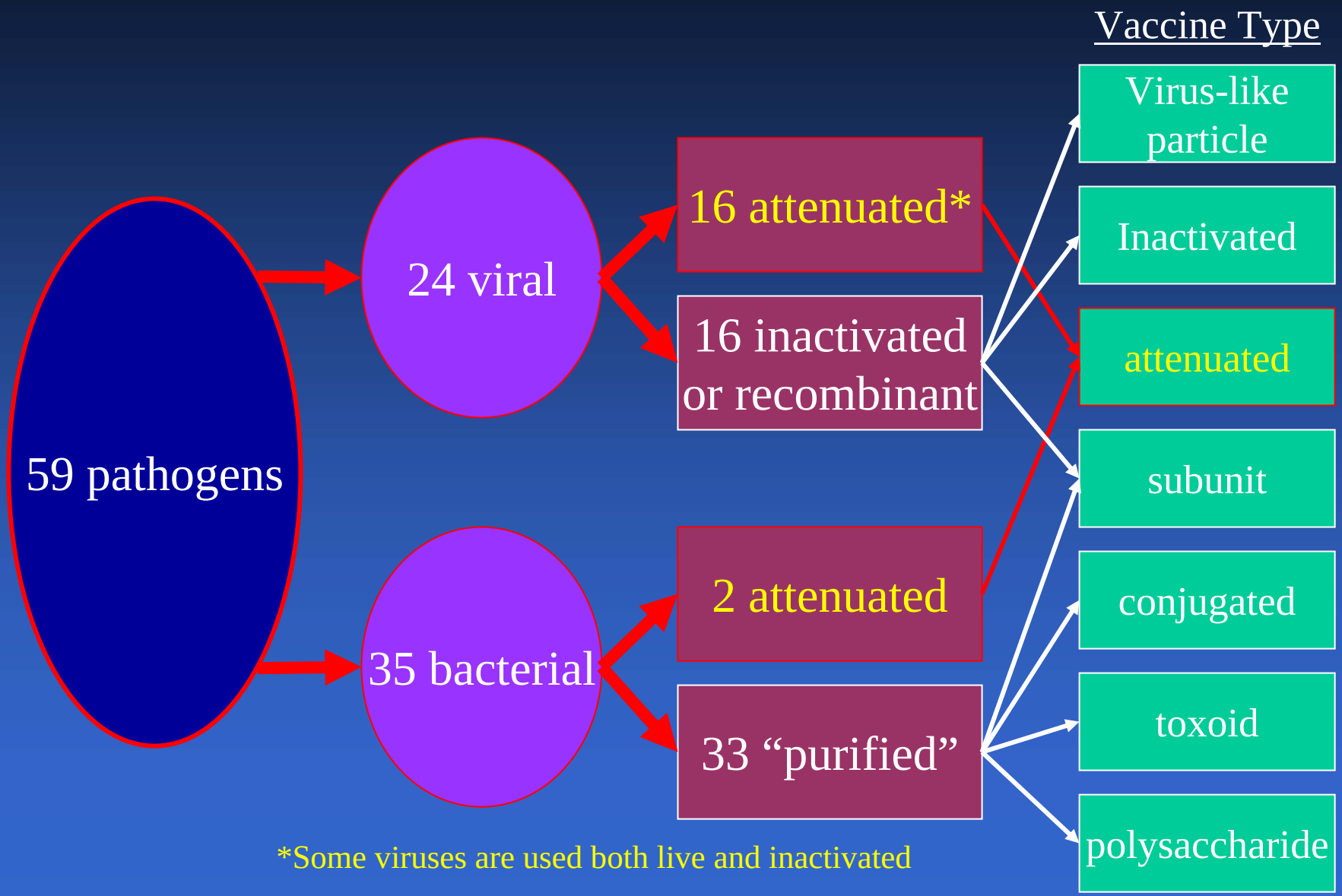
* Under review # inactivated or recombinant form available

A Limited Survey of Licensed Vaccines

- Licensed (and under review) vaccines cover 24 different pathogens (59 if all serotypes counted)
  - 14 viral (24 types)
  - 10 bacterial (35 types)
    - *C. diphtheriae*
    - *N. meningitidis* (4)
    - *S. pneumoniae* (23)
    - *B. anthracis*
    - *C. tetani*
    - *M. bovis**
    - *H. influenzae* B
    - *B. pertussis*
    - *S. typhii**
    - *V. cholerae*

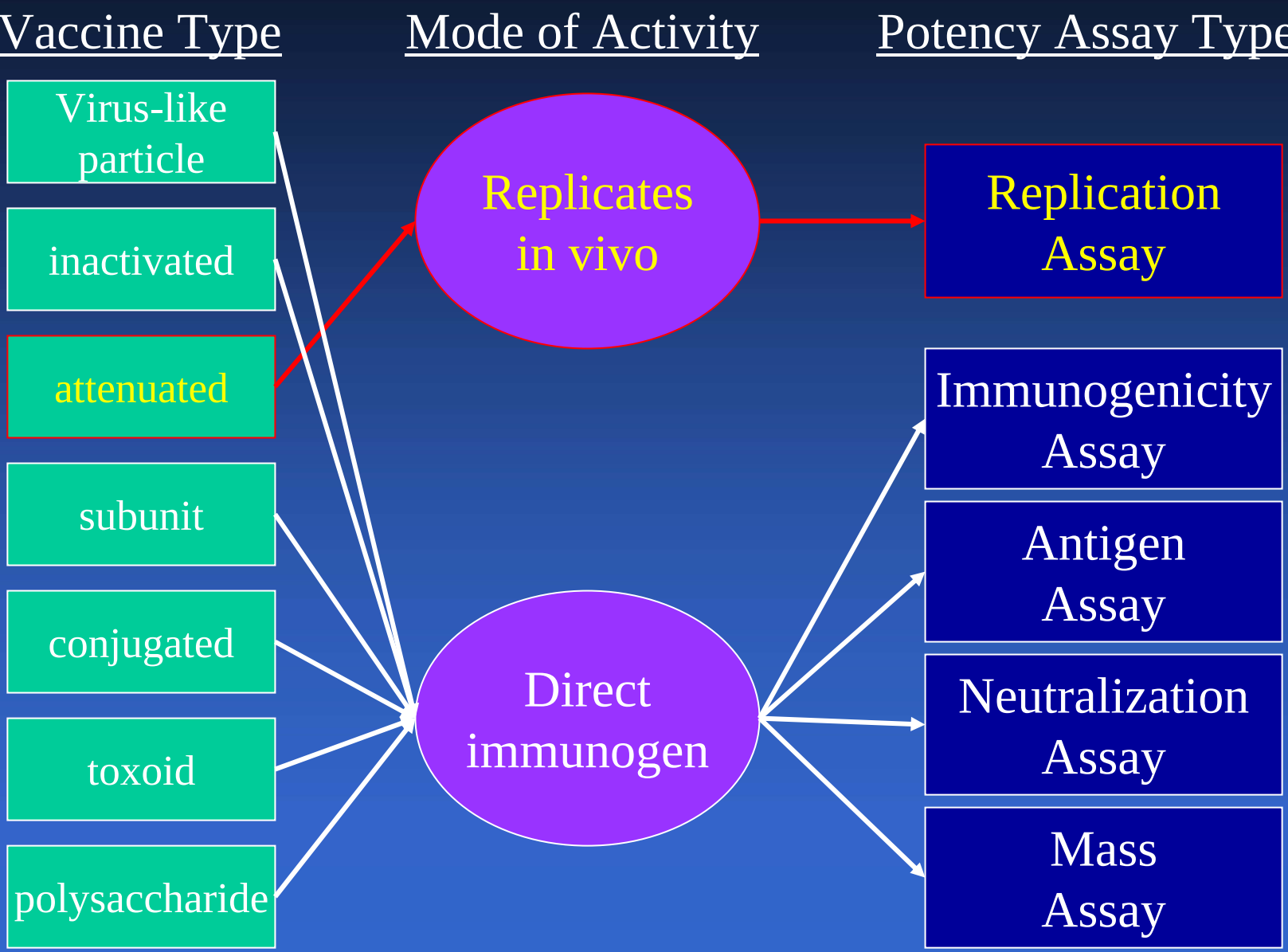
* attenuated forms available

Dissection of Vaccine Targets



*Some viruses are used both live and inactivated

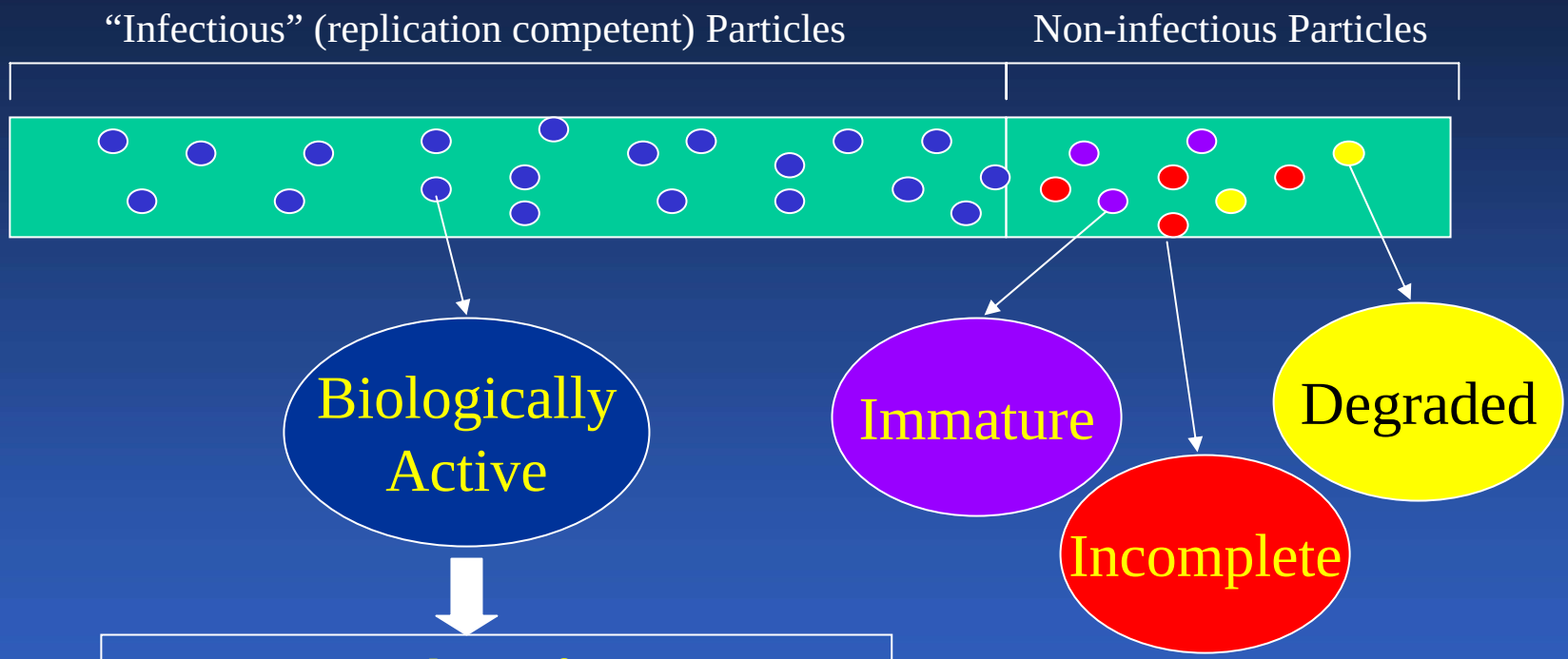
Type of Potency Assay Used Reflects Product Type



Replication Assays

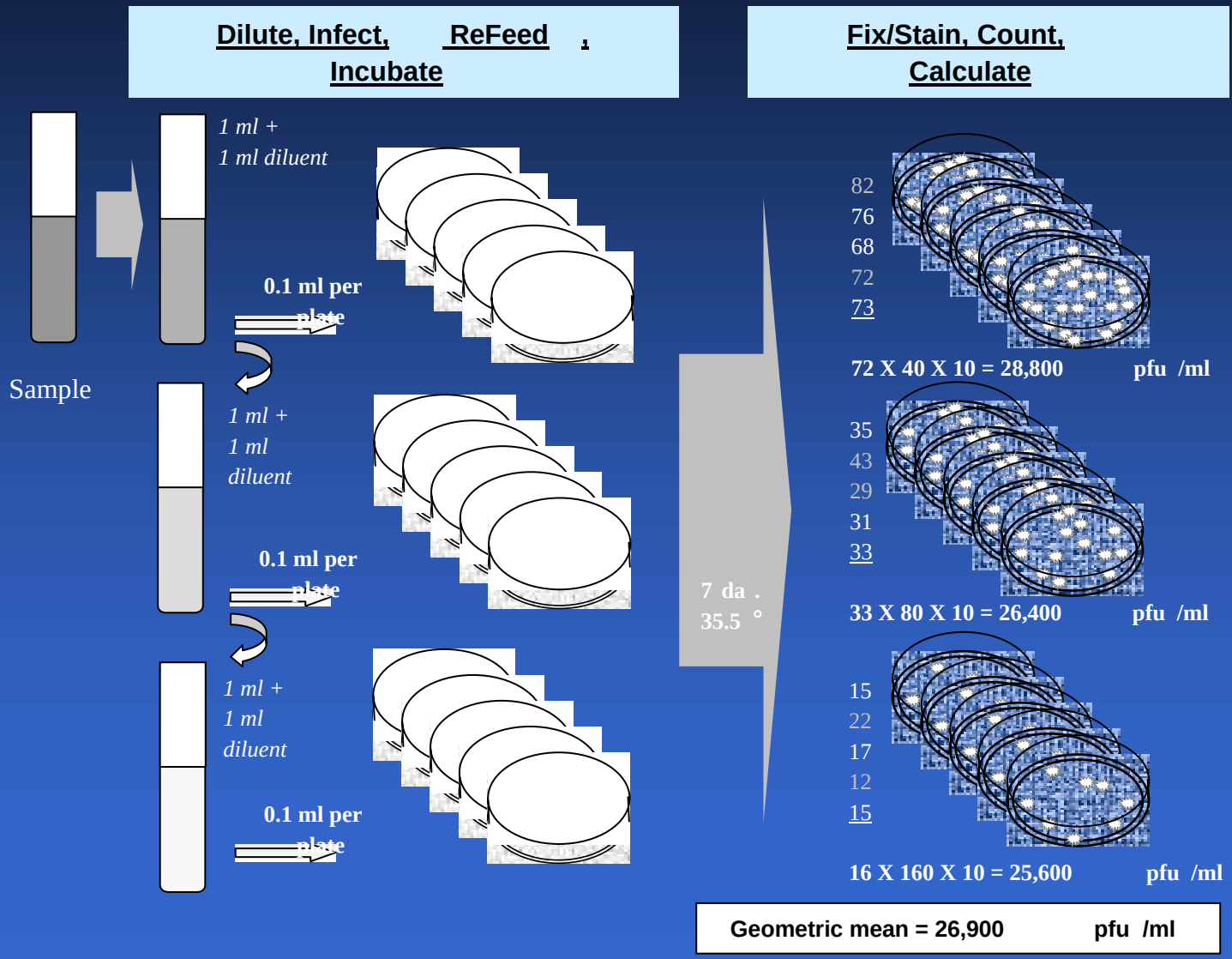
- Reflects the *in vitro* replication of the viable components (virus or bacteria) in a cell culture system
  - Virus replication typically reflects cytopathic effect (CPE) on (*i.e.* death of) cell substrate
    - Other approaches possible (to follow)
  - Bacterial replication typically reflects formation of visible colonies on a solid media substrate

In vitro Potency of Live Virus Vaccines

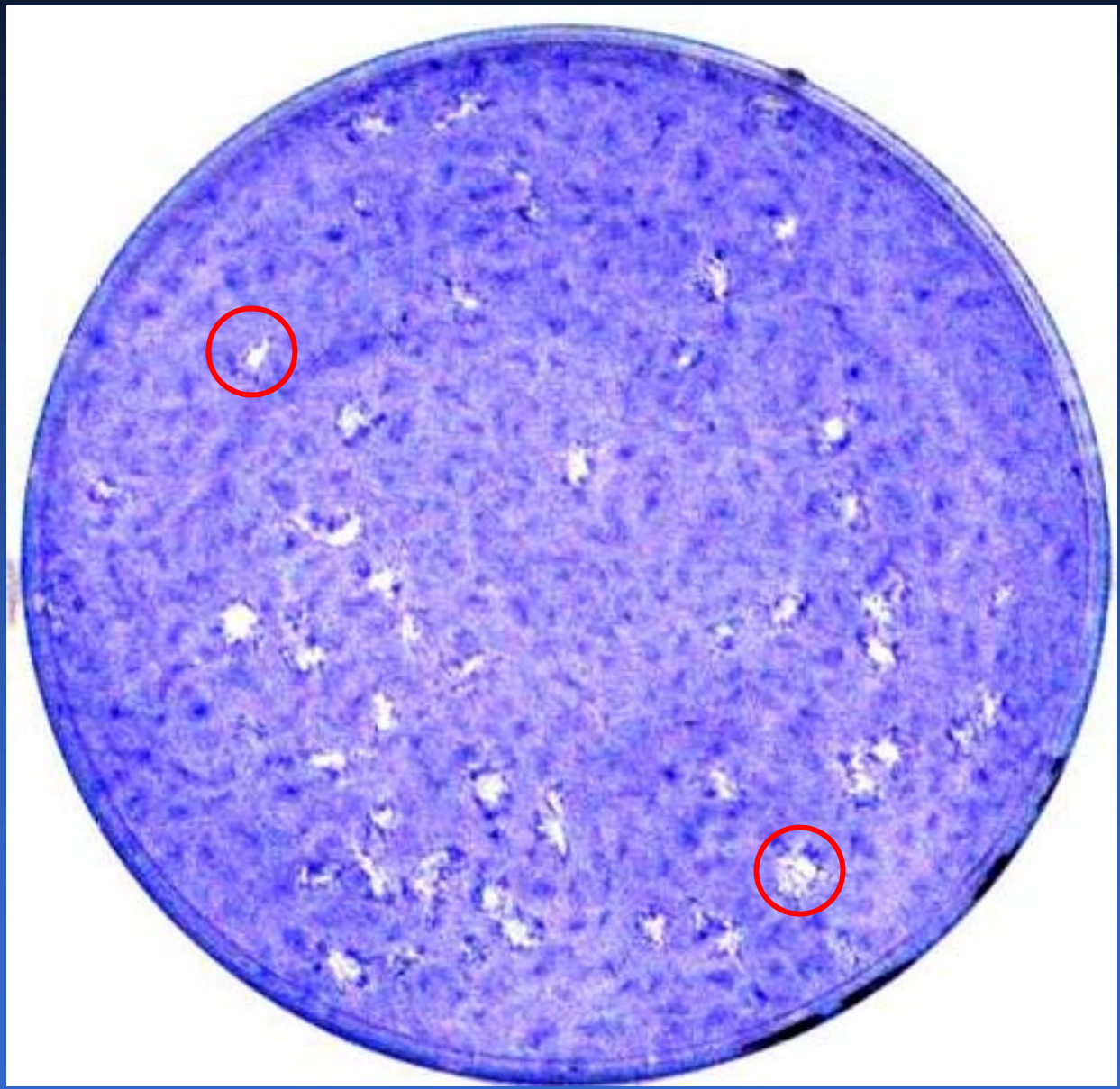


- quantitative plaque formation assay
- end-point dilution assay ($TCID_{50}$)
- virus neutralization assay
- quantitative PCR assay

Virus Replication: Quantitative Plaque Formation Assay



Varicella Plaque Assay: Infected plate



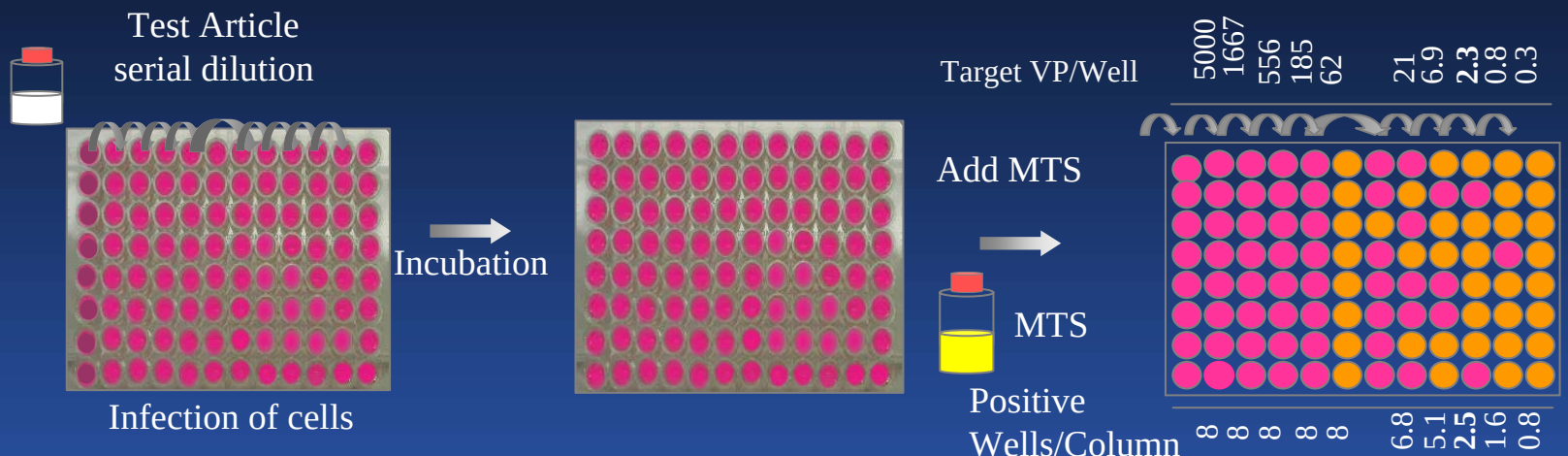
Benefits:

- Simple
- Easily transferable procedure

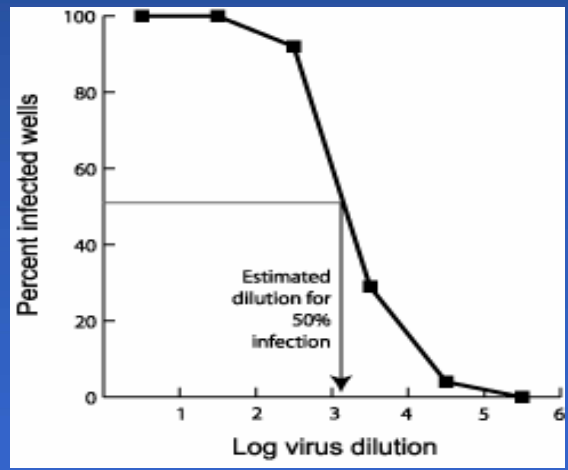
Drawbacks:

- Limited specificity
- Manually intensive
- Poor precision/ reproducibility
- Limited throughput

Virus Replication: $TCID_{50}$ Assay



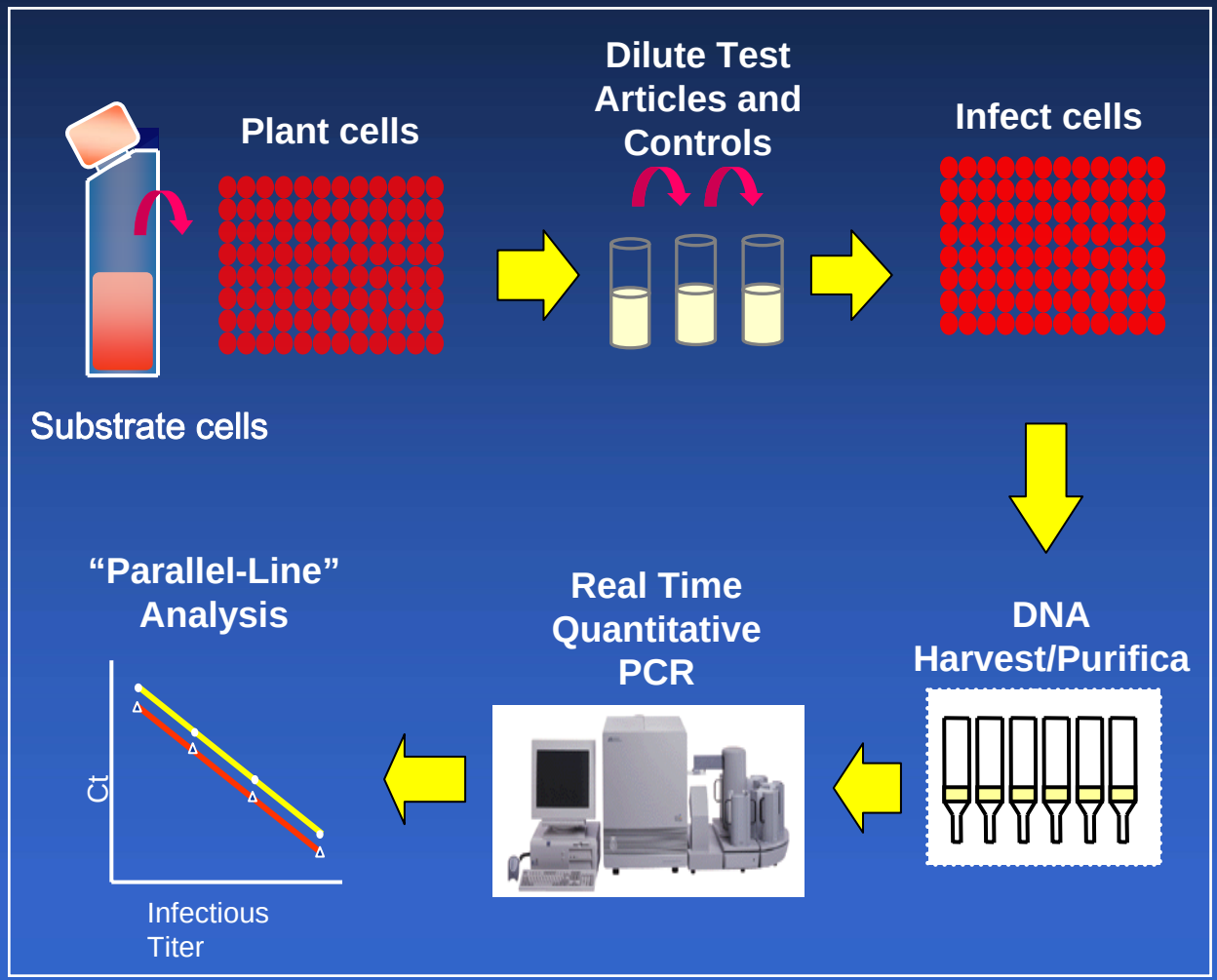
- Benefits
  - Semi-automated
- Drawbacks
  - Limited specificity
  - Wide virus dilution ranges needed
  - Poor precision/ reproducibility
  - Moderate throughput



Readout: $TCID_{50}$/mL

Root Assay Variability: ~60% RSD

Virus Replication: Quantitative PCR-based Potency Assay (QPA)



- Benefits:
  - Direct detection of viral (genome) replication
  - High throughput
  - Excellent specificity & sensitivity
  - Improved precision
- Drawbacks:
  - Costly system & reagents
  - New technology for QC lab

Bacterial Replication: Quantitative Colony Formation Assay

- Typically applying serial dilutions to solid-phase media, incubating, counting colonies
  - Bacterial equivalent of virus plaque assay
  - Specificity enhanced by use of selective media and evaluation of colony morphology
- Benefits and drawbacks similar to virus plaque assays

Immunogenicity Assay

- Reflects the *in vivo* biological response to the product in a relevant animal model
  - "Relevance" is relative
- Biomarker model
  - Assess biomarker (e.g. antibody levels) in pre- and post-vaccination sera
    - *e.g.* pertussis, Hib conjugate (no longer used)
  - Assess biomarker relative to reference standard
    - *e.g.* polio
- Challenge model
  - Neutralizing activity of serum from vaccinated animals in challenged animals
    - *e.g.* Diphtheria, tetanus

Immunogenicity Assay

- Benefits
  - Shows *in vivo* response
- Drawbacks
  - Relevance to predicting human response?
  - Requires continual supply of animals
  - Labor intensive
  - High variability

Antigenicity Assay

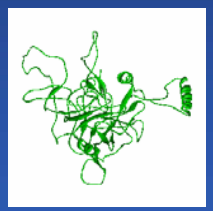
- Can be applicable to almost all vaccine components
  - Typically not used for “live” products
- Several formats to consider
  - Typical ELISA; extrapolation from std. curve
    - e.g. hepatitis A
  - IVRP; parallel line analysis, relative potency
    - e.g. hepatitis B, HPV
  - Competitive; four-parameter fit; relative potency
    - e.g. HPV (secondary assay)
  - Others (e.g. rate neph., rocket IE)
    - e.g. pneumococcal polysaccharides

Antigenicity Assay

- Benefits
  - Reflection of epitope content/integrity
  - Automatable
    - Relatively precise, moderate throughput
- Drawbacks
  - Presumed correlate of immunogenic potential
    - Sometimes bridged to immunogenicity assay (*e.g.* HPV)
  - Relevance to predicting human response?
  - Identifying/supplying reagents can be difficult

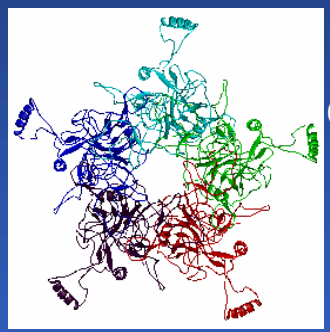
Structural Model of an HPV VLP

L1 protein
(55 or 57 kDa)



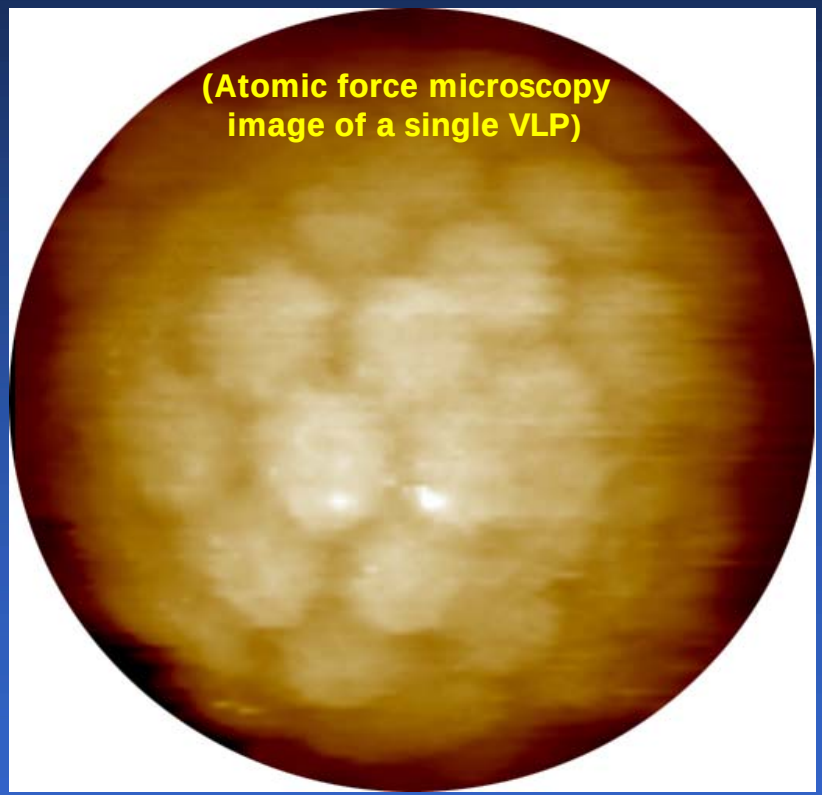
5 pL1

L1 Capsomere
(~280 kDa)



72 Capsomeres

Virus-Like Particle
(~20,000 kDa)



~3 nm

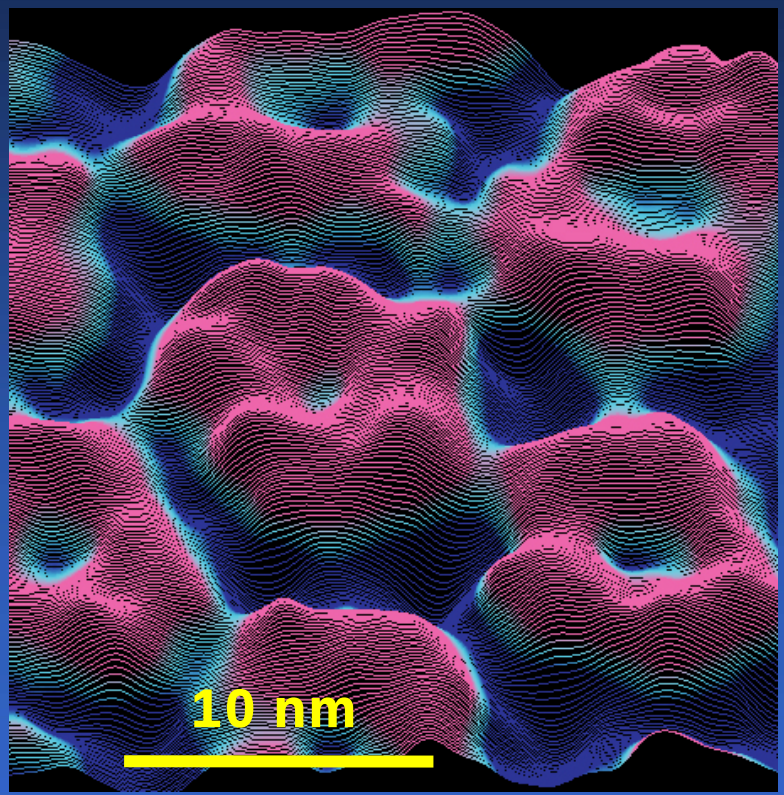
~10 nm

~60 nm

(Crystal structure coordinates courtesy of Prof. S. C. Harrison, Harvard University)

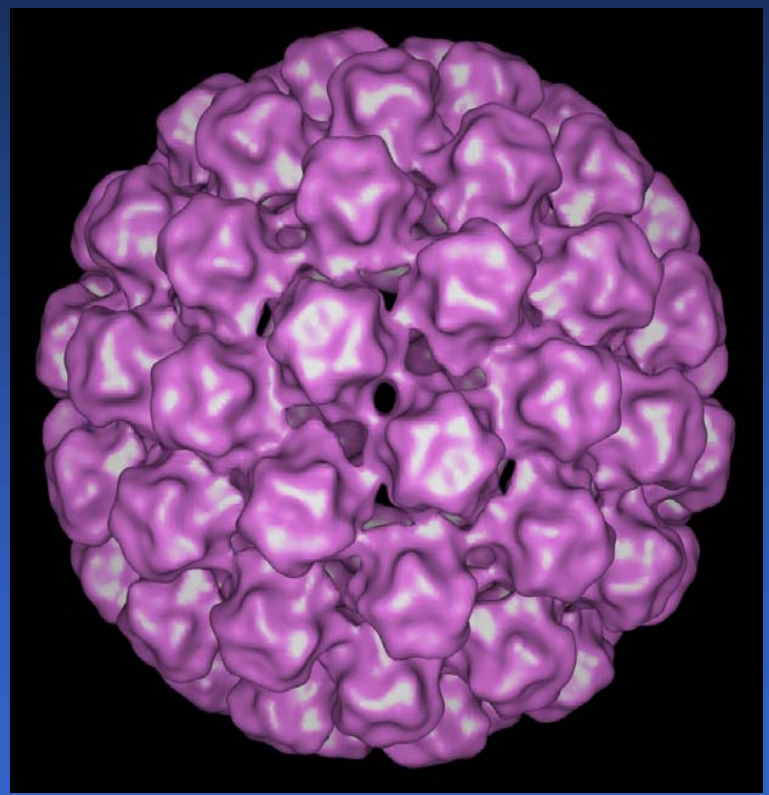
High-Resolution AFM Image of an HPV VLPs

HPV 16



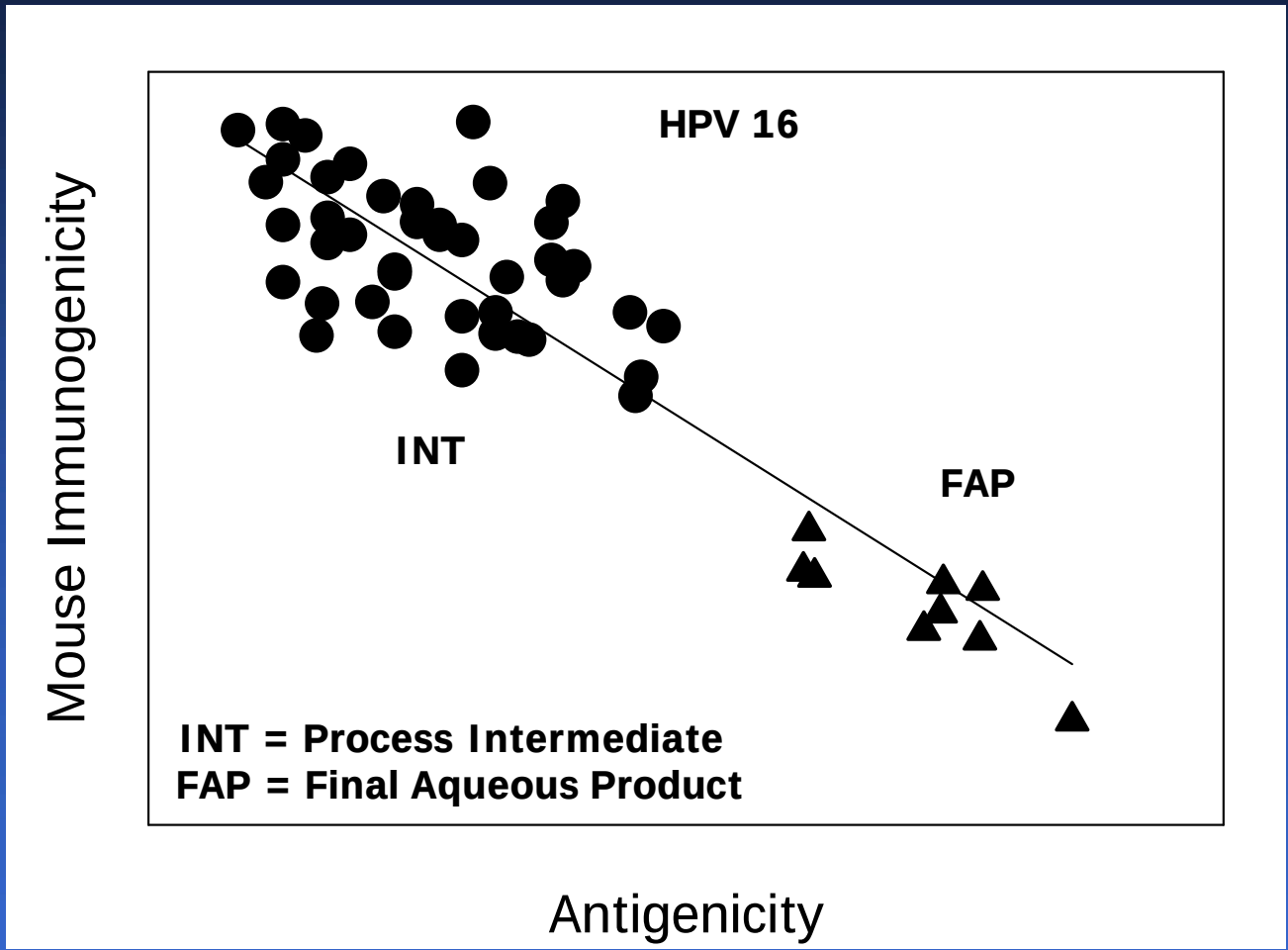
Morley & Y. Wang

HPV 18



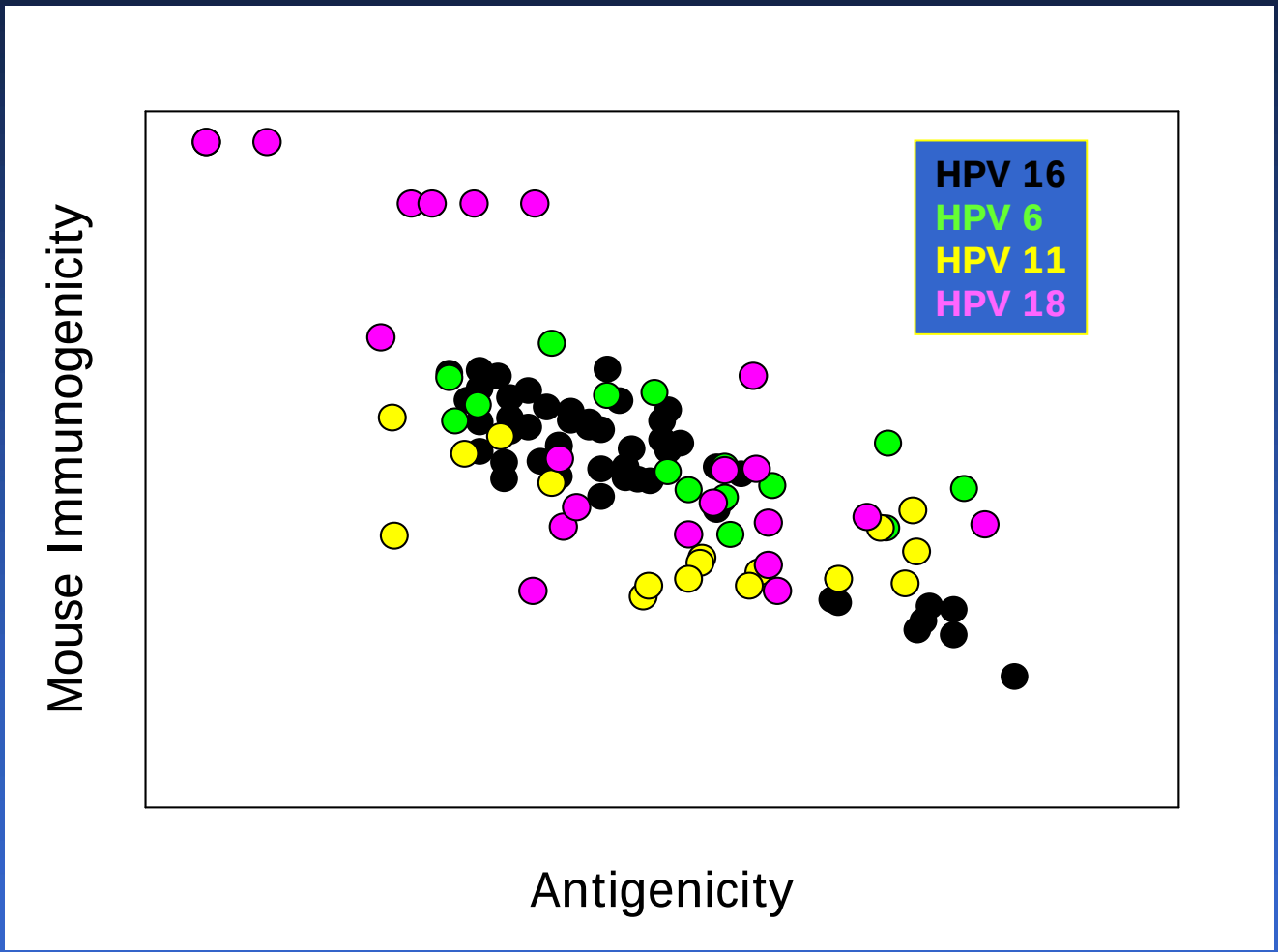
(20-Å Resolution *False-color* Image, Cryo-TEM)
Watson (AP-MSD), Duncan (MRL) & Potter (Scripps)

Correlation of Mouse Immunogenicity with Relative Antigenicity (IVRP)



F. Wang, Capen, Retzlaff, Schultz & Y. Wang

Correlation of Potency Measures for All Constructs



F. Wang, Capen, Retzlaff, Schultz & Y. Wang

Neutralization Assay

- Multiple approaches to “neutralization”
- Use specific antisera to block response
  - Replication assay
    - “Neutralize” replication
      - typically used for “identity” rather than potency
  - Immunogenicity assay
    - Immune response “neutralizes” pathogenic response
      - e.g. animal challenge assay (diphtheria, tetanus)
  - Antigenicity assay
    - “Neutralize” epitope binding
      - typically used for “identity” rather than potency

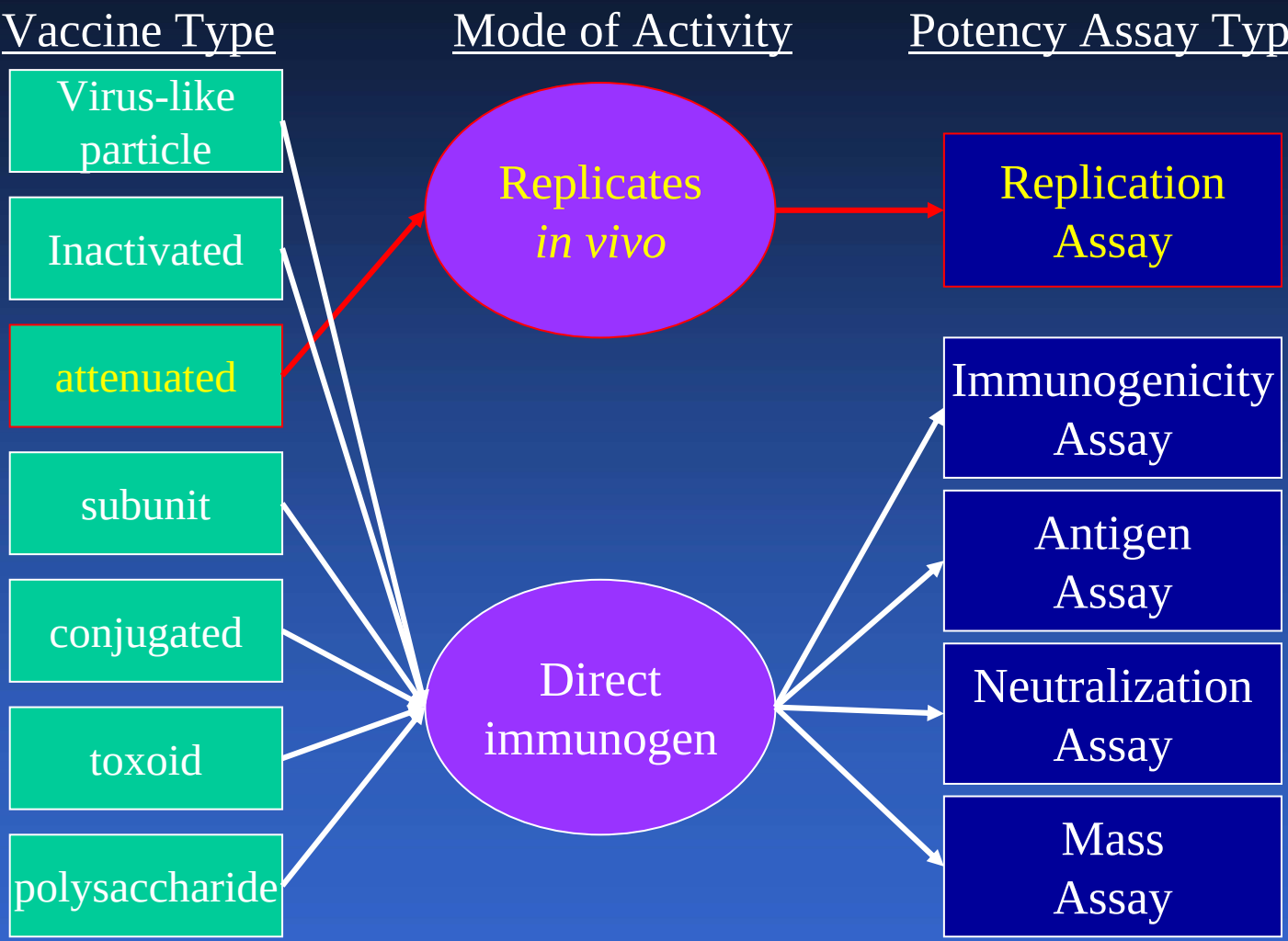
Mass Assay

- Assay that reflects “mass” of component in product (assumes potency-to-mass ratio is fixed)
  - Colorimetric assay
    - polysaccharide content (*S. typhii* Ps)
      - Secondary evaluation of MW and O-Ac content
    - protein assay (hepatitis B; secondary assay)
  - HPLC assay
    - polysaccharide content (Hib conjugate)
      - Secondary evaluation of MW and % conjugated

Mass Assay

- Benefits
  - Physico-chemical measurement
  - Limited variability
  - Automatable
    - Relatively precise, moderate throughput
- Drawbacks
  - High burden of proof that mass ~ potency
  - Relevance to predicting human response?

Choice of Potency Assay Depends on Product Type



New vaccine types (e.g. viral vectors, plasmids) will require consideration of new potency assay types.